

Poster presentation

ECOLITASTER: cellular biosensor

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Background

Our project consists of designing a cellular biosensor [1]. We use as a chassis an EnvZ- *E. coli* strain, and construct two different devices, a sensor and an actuator, assembled using OmpR-P as a standardized mediator. The sensor device contains a receptor protein and a synthetic two-component signal transducer, both implemented as new parts in the Registry of Standardized Biological Parts [2]. The device has been inspired on Hellinga's work [3], sensing TNT and other molecules by using a mutated periplasmic binding protein (PBP). Thus, our team thought in building a PBP that binds a vanillin molecule. The receptor protein has been computationally designed using a ribose binding protein as scaffold and finding the necessary mutations to change its ligand affinity towards vanillin, thus allowing the modified *E. coli* tasting flavors. Our computational methodology [4] can be used to test other molecules too. The synthetic two-component signal transducer was built by fusing the periplasmic and transmembrane parts of a Trg protein with an EnvZ kinase domain [5]. We expect this part to be very useful for future biosensors projects. When the PBP-vanillin complex binds Trg, an allosteric motion is propagated to the cytoplasmic EnvZ kinase domain, resulting in autophosphorylation and phosphate transfer to the OmpR transcription factor (OmpR-P), which then induces transcription of the pOmpR promoter. We use a synthetic genetic network as the actuator, which takes as input OmpR-P and as outputs GFP and RFP. At intermediate input levels, RFP will be expressed, whereas GFP is expressed proportionally to the

input to obtain a graded response if we superpose the outputs. This later device has been inspired on Weiss' work on pattern generation [6], but in this case we use a new two-regulator promoter to integrate the signals. This synthetic promoter is synergistically activated by two transcription factors cI and CRP (implementing an AND logic gate) [7], and it will be one of the first of its kind at the Registry [2]. We propose an extensive use of this type of

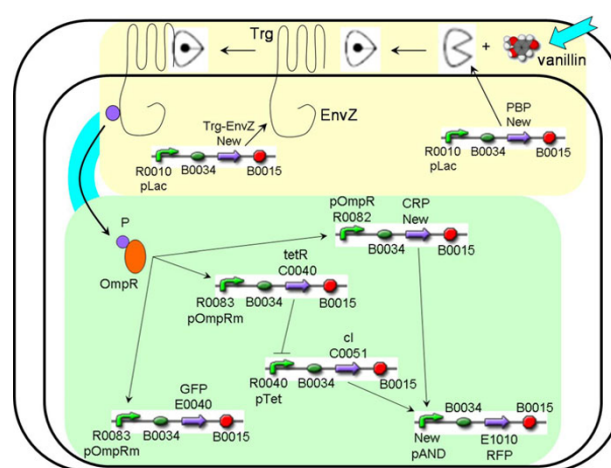


Figure 1
Biological scheme of the system using parts [2]. The yellow set shows the sensor device and the green one the actuator.

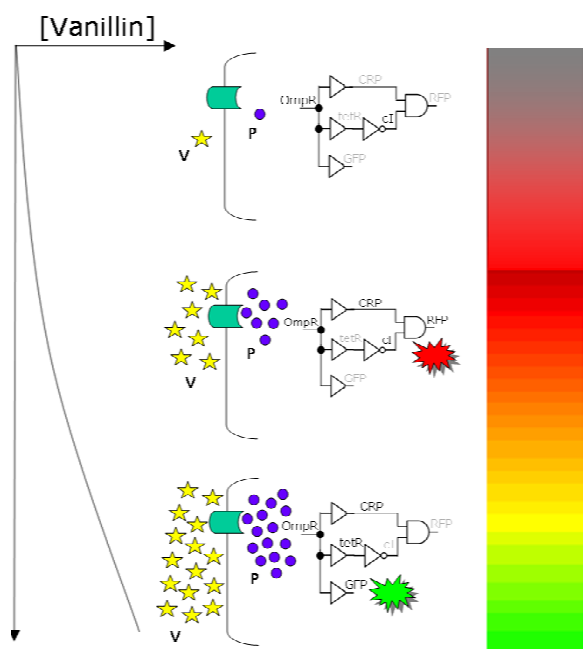


Figure 2
Mechanism of the system. Plot of the reporters according to the vanillin concentration.

promoters for future synthetic circuits, due to their small sequence size but sophisticated behavior.

Materials and methods

We have simulated our actuator device by using Matlab, and we have done a parametrical sensitivity analysis. On the other hand, we have obtained the sequence for the vanillin receptor by using a computational procedure [4]. We have used parts from the Registry [2] to implement our constructions by using standard assemblies [8]. Plasmids were derivated from pUC containing antibiotic resistances such as ampicillin or kanamycin. Furthermore, we have used DNA synthesis to obtain the CDS of the vanillin receptor. For the synthesis of the AND promoter [7], we have used DNA synthesis from oligos [9]. The CDS for the CRP and Trg were obtained from genomic PCR. The growth medium was LB at 37°C with the corresponding antibiotic. The reporters fluorescence has been measured by using FACS.

Results

We show the whole system (Figure 1) and its functional mechanism (Figure 2). Nowadays, we continue working on this project. All parts are already available. Hence, tasks are concentrated in assembling them. Then, we will characterize the biological devices.

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References

1. [\[http://www.intertech.upv.es/wiki\]](http://www.intertech.upv.es/wiki).
2. [\[http://parts.mit.edu\]](http://parts.mit.edu).
3. Looger LL, Dwyer MA, Smith J, Hellinga HW: **Computational design of receptor and sensor proteins with novel functions.** *Nature* 2003, **423**:185-190.
4. Jaramillo A, Wernisch L, Hery S, Wodak SJ: **Folding free energy function selects native-like protein sequences in the core but not on the surface.** *Proc Natl Acad Sci USA* 2002, **99**:13554-13559.
5. Baumgartner JW, Kim C, Brissette RE, Inouye M, Park C, Hazelbauer GL: **Transmembrane Signalling by a Hybrid Protein: Communication from the Domain of Chemoreceptor Trg That Recognizes Sugar-Binding Proteins to the Kinase/Phosphatase Domain of Osmosensor EnvZ.** *J Bacteriol* 1994, **176**:1157-1163.
6. Basu S, Gerchman Y, Collins CH, Arnold FH, Weiss R: **A synthetic multicellular system for programmed pattern formation.** *Nature* 2005, **434**:1130-1134.
7. Joung JK, Koeppe DM, Hochschild A: **Synergistic activation of transcription by bacteriophage λ cI protein and *E. coli* cAMP receptor protein.** *Science* 1994, **265**:1863-1866.
8. Endy D: **Foundations for engineering biology.** *Nature* 2005, **438**:449-453.
9. Rouillard JM, Lee W, Truan G, Gao X, Zhou X, Gulari E: **Gene2Oligo: oligonucleotide design for in vitro gene synthesis.** *Nucleic Acids Res* 2004, **32**:W176-W180.

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